

Catching evaders

Citation for published version (APA):

Denisov, S. S. (2019). *Catching evaders: structure elucidation and molecular mechanisms of tick salivary proteins*. [Doctoral Thesis, Maastricht University]. ProefschriftMaken.
<https://doi.org/10.26481/dis.20191218sd>

Document status and date:

Published: 01/01/2019

DOI:

[10.26481/dis.20191218sd](https://doi.org/10.26481/dis.20191218sd)

Document Version:

Publisher's PDF, also known as Version of record

Please check the document version of this publication:

- A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher's website.
- The final author version and the galley proof are versions of the publication after peer review.
- The final published version features the final layout of the paper including the volume, issue and page numbers.

[Link to publication](#)

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license above, please follow below link for the End User Agreement:

www.umlib.nl/taverne-license

Take down policy

If you believe that this document breaches copyright please contact us at:

repository@maastrichtuniversity.nl

providing details and we will investigate your claim.

Chapter 7: Summary

Summary

This thesis deals with study of tick cysteine-rich chemokine binding proteins Evasin-3 and Evasin-4 by several biophysical techniques such as NMR spectroscopy, X-ray crystallography, and surface plasmon resonance biosensor analysis. In the **Chapter 1** known approaches of disulfide mapping in cysteine-rich peptides are reviewed. The short review of tick cysteine-rich proteins is given in the second part of this chapter.

Chapter 2 establishes a novel technique of mapping disulfide bond connectivity using selenocysteine scanning (SecScan) and NMR spectroscopy. It has been shown that single substitution of cysteine by selenocysteine causes selective perturbations of α and β chemical shifts across the mixed S-Se bond. This approach was validated on cysteine-rich peptide and proteins with known disulfide connectivity – μ -conotoxin KIIIA, Kalata B1, and bovine pancreatic trypsin inhibitor (BPTI). SecScan was applied to unravel disulfide network of a tick saliva protein Evasin-3. Obtaining three synthetic truncated Evasin-3 variants with single Cys/Sec substitution we showed presence of inhibitory cystine knot in the structure of this protein. Applicability of SecScan has been further expanded to expressed proteins. Using *E.coli* expression system with the reprogrammed genetic code, disulfide connectivity of a tick protein BSAP1 has been established.

Chapter 3 describes a solution NMR spectroscopy and solid phase peptide synthesis approach to study the chemokine-binding tick protein Evasin-3 and its complex with CXCL8. The high-resolution structure was obtained for the Evasin-3 core region and used in molecular docking with CXCL8. We showed that Evasin-3 disrupts CXCL8 dimer and prevent its binding to GAGs. Having elucidated structure of Evasin-3, two novel CXCL8 binders were derived from the Evasin-3 sequence. Both compounds showed nM affinity to CXCL8 and effectively inhibit CXCL8-induced migration of polymorphonuclear neutrophils (PMN).

Chapter 4 reports on application of a fluorescently labelled Evasin-3 variant for imaging of CXCL1 deposited on endothelium cell wall in inflamed arteries. Solution NMR spectroscopy was used to prove that Evasin-3 binds CXCL1 but leaves one of GAG binding sites unoccupied and vacant for GAG binding. The fluorescent label was incorporated to Evasin-3 site-selectively during solid phase peptide synthesis and was shown to not affect affinity to CXCL1. Obtained fluorescently labelled Evasin-3 was successfully applied for imaging CXCL1 in human microvascular endothelial cells and on activated endothelium of mouse carotid arteries.

Chapter 5 addresses the study of Evasin-4 by NMR spectroscopy and X-ray crystallography. Recombinantly expressed Evasin-4 was crystallize and its structure resolved by X-ray crystallography. Evasin-4 crystallizes in dimeric form and retains the similar to Evasin-1 three-dimensional fold. Formation of the complex of Evasin-4 with CCL5 was followed by solution NMR spectroscopy. We showed that Evasin-4 binds N-terminal region of CCL5 disrupting CCL5 dimer. Moreover, Evasin-4 binding to CCL5 is not affected by presence of high excess of CXCL4 with which CCL5 form biologically active heterodimers.